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# DELAYED PISTIL SENESCENCE CAUSES AN OVERLAP IN PROTOGYNY IN 'TONNAGE' AVOCADO (*PERSEA AMERICANA* MILL.)

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**ABSTRACT**

Pistils were hand pollinated with self pollen at the end of the male opening in 'Tonnage' avocado *(Persea americana* Mill.). After 24 hours, flowers were collected and pistils were observed for pollen tube growth using a fluorescent technique. In 85% of the pistils examined, self pollen germinated on the stigma. In 37.5% of the pistils observed, selfed pollen tube growth had not reached midway down the style. In 25% of the pistils, selfed pollen tubes grew halfway down the style. In 22.5% of the pistils, selfed pollen tubes were observed at the base of the ovule. 52.5% of pistils had a small amount of callose, while 47.5% of the pistils observed were callose free. This study is the first known record of selfed pollen tube growth in 'Tonnage' avocado at the end of the male opening.

**INTRODUCTION**

Nirody (1922) first reported avocado flowers, *(Persea americana* Mill.), to be protogynous. Protogyny is a form of dichogamy; *i.e.,* a temporal separation of male and female functions within a single flower where the female phase is presented before the male phase, thus minimizing selfing and promoting cross-pollination. Stout (1924) described two morph types. Flowers of type (A) cultivars first opened as functional females in the morning hours. These flowers subsequently closed at midday and reopened again the following afternoon as functional males. Flowers of type (B) cultivars opened as functional females in the afternoon and reopened as males the following morning. Pollen was available for transfer upon dehiscence of the anther valves that expose pollen in spoon-shaped pockets during the male opening.

Florida avocado growers usually interplant complementary types in groves in order to increase the likelihood of cross-pollination. High fruit set, however, has been recorded in solid plantings (Robinson, 1933), and in isolated trees (Cintron, 1947). Further evidence of selfingis reported from isozyme electrophoresis studies (Degani *et al.,* 1986; Torres and Bergh, 1978). In reciprocal matings between the variety 'Ettinger' (the female parent) and 'Rosh Hanrika' (the male parent), 80% of the progeny were shown to be a result of self-pollination (Degani and Gazit, 1984).This study did not, however, give reasons why and how selfing occurred. *Persea americana* Mill, is generally considered to be self-compatible. However, if there is no overlap in protogyny, selfing is not possible and fertilization can occur only from out-crossing.

Although self-pollination is thought to occur in avocado, few studies have described in detail the path of selfed pollen tubes to the ovary. Sedgely (1977) described selfed pollen tube growth in the variety 'Fuerte' as being slow and retarded by callose production in the pistil. No selfed pollen arrived at the base of the ovary 24 hours after self-pollination in this study. Sedgely also suggested that the presence of callose in the pistil during the male opening rendered the pistil nonfunctional and that fruit set from selfed pollination was impossible. Self-pollination can be successful in avocado flowers only if pollen comes in contact with a stigma that is still receptive at the end of the male opening on a single tree. Such a condition occurs when there is an overlap in protogyny within the avocado flower.

In a large number of Florida-grown avocado cultivars, the pistil appears shriveled at the time of the male opening. However, if the pistil is still viable by the end of the male opening and callose production is minimal in the pistil at the time of self-pollination, fertilization may occur in these flowers. Davenport (1986) suggested the evolution of a "fail safe" mechanism, to ensure fertilization during variable environmental conditions, as one reason why selfing has been selected in some varieties. If these conditions occur, then selfing would be important in the fertilization process. This indicates that there may be a "window of opportunity" for the breakdown of dichogamy, possibly stimulated by low fruit set, from year to year.

This paper presents the first evidence of selfed pollen tube growth in the pistil of ‘Tonnage’ flowers 24 hours after self-pollination at the end of the male opening.

## MATERIALS AND METHODS

Flower buds from 'Tonnage' avocado (Type B) were chosen at random, marked with a waterproof pen, and enclosed in fine mesh organdy bags early in the morning. Care was taken to remove all older flowers and very young buds from an inflorescence prior to bagging. When flowers opened as females in the afternoon, any remaining buds that had not opened were removed. During this female opening, the pollen had not dehisced and the anthers lay flat. Each flower was then emasculated, making sure no damage was done to the pistil. The flowers were then rebagged. This ensured that no self pollen or cross pollen was allowed to come in contact with the receptive stigma. At the end of the male opening the next morning, flowers were hand-pollinated with self pollen using pollen from flowers of the same tree. Forty marked flowers were self-pollinated, and the inflorescences rebagged for 24 hours.

After 24 hours, flowers were collected and fixed in FAA (1:1 ethanol:acetic acid). The entire pistil was dissected from each flower, cleared with 5M NaOH for 24 hours, rinsed with distilled water, and stained with decolorized aniline blue (Martin, 1959). Pollen tubes were observed using a Leitz Dialux microscope equipped with a Lietz epifluorescence attachment A (Excitation filter, BP 340-380; suppression filter BP 410-410-580). Callose present in pollen tubes emits a yellowish green fluorescence and can easily be seen in gynoecial tissue.

## RESULTS

Of the total number of self-pollinated stigmas, 85% of the pistils showed pollen

germination after self-pollination in the male opening. Pollen tubes in 37.5% of pistils examined did not grow as far as midway down the style. 25% of the pistils observed showed pollen tubes growing half the way down the style, and in 22.5% of pistils, pollen tubes were observed growing to the base of the ovule. In 52.5% of pistils examined, a small amount of callose was present, while in 47.5% of pistils there was no callose present 24 hours after self-pollination. No pollen tube growth occurred in 15% of the pistils after hand pollination. Callose production appeared delayed in the gynoecial tissue. Since inflorescences were bagged before buds opened, any pollen tube growth observed would have been from selfed pollen.

Table 1. *Selfed pollen tube growth 24 hours after hand pollination in 'Tonnage' avocado*

| % of pistils showing no germination | % of pistils showing germination on the stigma | % of pistils showing pollen tube growth not reaching 1/2 way down the style | % of pistils showing pollen tube growth 1/2 way down the style | % of pistils showing pollen tube growth at base of ovule |
|---|---|---|---|---|
| 15 | 85 | 37.5 | 25 | 22.5 |

Figure 1-4 show pollen tube growth in self-pollinated pistils of ‘Tonnage’ flowers. Fluorescence of pollen tube was yellowish green.

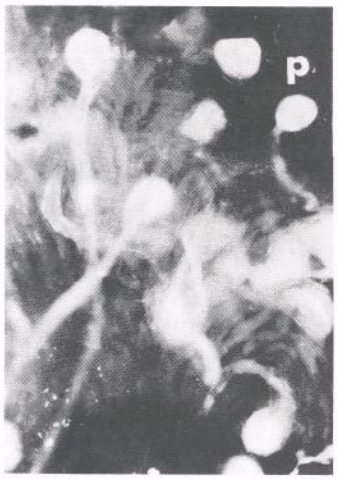


*Figure 1*

*Fig. 1* shows germination of selfed pollen grains on the stigma.


*Figure 2*

*Fig. 2* shows selfed-pollen tube growth halfway down the style.

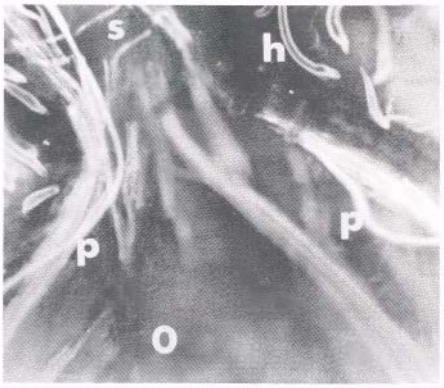


*Figure 3*

*Fig. 3* shows pollen tube growth at the base of the style. Selfed pollen tubes are seen growing around the ovary on both sides. At this stage there was no obvious leader pollen tube.

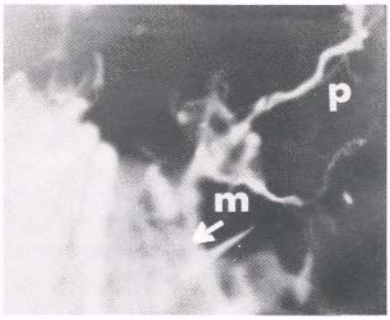


*Figure 4*

*Fig. 4* shows a selfed-pollen tube in the micropylar region of the ovule.

## DISCUSSION

This study is the first to show selfed pollen tubes growing down the pistil in the 'Tonnage' avocado. It is important to note that hand pollinations were done at the end of the male opening. Selfed pollen tube growth recorded 24 hours after hand pollination, at the end of the male opening, is a remarkable find and gives more credence to the hypothesis that there is an overlap in protogyny in 'Tonnage' avocado. This overlap would provide a large window of opportunity for selfing. Any mutation which renders the pistil viable at the end of the male opening may be selected in nature if crossing is limited or not successful. It has not been proved, but it is speculated here that low fruit set in previous years may be the primary reason for the delay in pistil senescence in 'Tonnage' avocado. Selection may be acting on those varieties that have a "loosely" regulated gene for pistil viability. This is not to say that all varieties of avocado have the ability to self, as it is obvious that some varieties are obligate outcrossers; but there is some indication that some varieties like 'Tonnage' may be undergoing a shift in floral phenology to ensure fertilization. Lack of pollinators in some areas may also cause a shift toward selfing in some varieties. If fruit set is pollinator limited, then hard and fast outcrossers would be at a disadvantage, as fruit set would be very low when pollinators are scarce.

Pistils of 'Tonnage' avocado flowers are not shriveled at the end of the male opening, allowing selfed pollen to germinate and grow down to the micropylar region of the ovule. Since survival to the next generation is paramount, then selfed fruits could carry the gene for prolonged pistil viability to the next generation. If fruits were inferior, then this activity would be not be selected in the next generation. If the fruits of 'Tonnage' avocado are high in quality, then this avocado variety would be valuable to growers. Because of this novel find in ‘Tonnage’ avocado, further pollen tube studies should be undertaken to obtain data on whether other avocado varieties are selfing but have not been reported.

This preliminary study has presented novel information for 'Tonnage' avocado. It is strongly suggested that further experimental work be done on this variety, especially the collection of data on the quality of fruit set from selfs and crosses. Fruit set was abundant in ‘Tonnage’ (personal observation), and pistil viability appeared to be the limiting factor for fruit set. Fruits from ‘Tonnage’ avocado should be randomly picked to assess how many flowers set fruit from selfing or crossing, in nature. Pollen tube growth rates for selfs and crosses should be determined. Only these types of studies can discern the true nature of avocado pollination and fertilization and give insight to the dynamics of protogyny and selfed pollen tube growth in *Persea americana* Mill.

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